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Therapeutic Effects of Rocket Leaves (*Eruca sativa*) against Hepato-Renal Toxicity Caused by Xylene in Wistar Rats

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ABSTRACT

Nutritious foods like vegetables and fruits have therapeutic potential in treatment of many human diseases. Several bioactive compounds have been extracted from plants and fruits, especially antioxidants which aid protect against oxidative stress. This study was conducted on *Albino Wistar* rats to investigate the antioxidant effect of *Eruca sativa* on the hepatic and renal profile damaged by xylene. Seventy rats were divided into seven equal groups and treated by gavage for 30 days as follows: Control group (C), group (CO), positive control group (RE) was received 350 mg / Kg bw of *Eruca sativa* aqueous extract (ESAE), 2 toxic groups X1 and X2 were treated with xylene at two doses 400 and 800 mg / Kg bw and 2 combination groups REX1 and REX2 were treated with ESAE (350 mg/kg bw) combined with 400 and 800 mg/Kg xylene respectively. The results revealed that oral treatment with xylene (X1 and X2) caused hepatic and renal dysfunction, which was manifested by a significant elevation of AST, ALT, ALP, urea, uric acid and creatinine plasma levels. Additionally, a significant decrease in GSH levels and GPx activity accompanied with an increase in MDA levels were noted due to the exposition of xylene. However, animals that received ESAE with xylene (REX1 and REX2) showed an adjustment of these perturbations, by a significant decrease in the levels of AST, ALT, ALP, urea, uric acid, creatinine and MDA. As well as an increase in GSH levels and GPx activity compared to X1 and X2 groups. The histological profile of toxic groups showed pathological changes in liver and kidney tissue were characterized by sinusoidal and tubular dilatation, hemorrhage, hepatocyte necrosis and glomerular degeneration, the effect of ESAE was effective in modified these damages into semi normal.To conclude, xylene administration induced hepatotoxicity and nephrotoxicity in wistar rats, while the combination of this solvent with rocket aqueous extract (*Eruca sativa*) attenuated this toxicity thanks to its antioxidant property.

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Key words
***Eruca sativa*, Xylene, Hepatotoxicity, Nephrotoxicity, Oxidative stress, Antioxidant, Rats**

INTRODUCTION

The liver is the responsible organ for the majority of toxic chemical and drug metabolism, and is therefore the primary target organ for numerous organic solvents (Malaguaenera *et al*., 2012). Metabolic waste excretion is principally done through the kidneys, where they can become vulnerable to toxicity (Brater and Hall, 2000). Xylene is one of the top 30 chemicals manufactured in the United States in terms of volume (ATSDR, 2007). It is a mixture of the isomers (Meta, ortho and para-xylene) with variable percentages of ethylbenzene. It is used as a solvent, cleaning agent, and notably in the printing, paint, rubber and leather industries (Le Floch *et al*., 2012). Main effects of xylene exposure affect the nervous system by all routes of exposure, the respiratory tract by inhalation, and at higher oral exposure levels, causes liver and kidney effects (ATSDR, 2007). Organic compounds toxicity is mainly manifested through the generation of reactive oxygen species (ROS) and consequent inhibition of antioxidant enzymes (Chen *et al*., 2000). Studies have revealed that xylene causes oxidative damage in the body through the production of ROS (Salimi *et al*., 2017).

Supplementation of exogenous antioxidants or strengthening the body endogenous antioxidant defenses is a promising means of combating the undesirable effects of oxidative stress induced by ROS. Plants possess an innate capacity to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating oxidative damage (Kasote *et al*., 2015). *Eruca sativa* or rocket plant is an annual herbaceous plant, belongs to the Brassicaceae family and mainly originates from Mediterranean countries and Western Asia (Vieira *et al*., 2015). It has been recognized as a rich source of beneficial phytochemicals for health such as vitamins, carotenoids, fibers, minerals, glucosinolates, isothiocyanates, flavonoids and phenolic

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compounds (Garg and Sharma, 2014). Rocket plays an important role in several biological activities, including antioxidant, anticancer, antifungal, antibacterial (Kim *et al*., 2004), anti-inflammatory (Yehuda *et al*., 2009), hepatoprotective and other activities (Al qasoumi, 2010).

The main objective of our work is to evaluate the antioxidant effect of aqueous extract of rocket (*Eruca sativa*) against xylene-induced liver and kidney damage in male wistar rats.

MATERIALS AND METHODS

Animals materials

This study was carried on 70 adult male rats of the *Albino wistar* strain with average body weight of 200–250 g, and purchased from the Pasteur institute, Algiers (Algeria). These rats were hosted in the biology department animal facility (University Badji Mokhtar-Annaba) and were subjected to an adaptation period of 30 days, in plastic cages, where they were provided with standard diet and tap water *ad libitum*.

Chemical material

For our study we chose xylene (96% purity) was supplied by Sigma-Aldrich (St. Louis, MO, USA). It is a monocyclic compound of the aromatic hydrocarbon family with the chemical formula: C_8H_{10}.

Plant material and preparation of aqueous extract

Plant material is consists rocket leaves (*Eruca sativa*), it belongs to the Brassicaceae family and originates from the Mediterranean region but also widely cultivated all over the world.

The leaves were obtained in January 2020 from the local market of Annaba (Algeria). This leaf part of the plant was dried at room temperature and protected from direct sunlight, in order to preserve the maximum molecules integrity. Then the leaves were crushed into a fine powder using an electric mixer. The powder was stored in dark glass to protect it from light and mold. The extraction method that we used is maceration, the leaves powder was extracted for 24 h in distilled water and filtered through a compress.

Experimental design

Wistar rats were divided into 7 groups (n= 10 in each group), the treatment was performed orally, daily for 30 days as follows: Group C (control group): received tap water; Group CO: treated with 0.3 ml of corn oil; Group RE (positive control group): treated only with ESAE at 350 mg/kg bw; Group X1: treated with the 1st dose of 400 mg/Kg bw of xylene diluted in corn oil; Group X2: treated with the 2nd dose of 800 mg/Kg bw of xylene diluted in corn oil; Group REX1: treated with ESAE (350 mg/kg bw) combined with the 1st dose of xylene (400 mg/kg bw) and Group REX2: treated with ESAE (350 mg/kg bw) combined with the 2nd dose of xylene (800 mg/kg bw).

Sample collection

At the end of the treatment period, blood was immediately collected in heparin tubes, which were centrifuged at 3000 rpm for 10 min, and then the plasma was stored at -20 °C together with the liver and the kidney till further analysis (for biochemical and oxidative stress parameters).

Biochemical assays

Plasma levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, uric acid, and creatinine were carried in the Clinical Chemistry Laboratory using an automated random access clinical chemistry analyzer (Xl-600, ERBA Diagnostic Mannheim GmbH, Germany). They were performed by kits provided by Spinreact, Spain.

Oxidative stress assays

Liver and kidney were used for the determination of the malondialdehyde level (MDA) according to the method of Ohkawa *et al*. (1979), reduced glutathione level (GSH) by the method of Weekbeker and Cory (1988), glutathione peroxidase (GPx) activity, by the method of Flohe and Gunzler (1984), and total protein concentration by the method of Bradford (1976).

Histopathology assays

For histological studies, portions of liver and kidney tissue were fixed in 10% buffered formalin solution, dehydrated in an ascending graded series of alcohols (70% –100%) and cleared in xylene before embedding in molten paraffin wax. Sections of 4–5 µm thickness were cut by microtome and then stained with haematoxylin and eosin (H & E) according to Hould (1984).

Statistical analysis

All test results carried were expressed as mean±SEM using Prism7 software. Data comparisons between the different treatment groups were performed by the one-way ANOVA test and Tukey's multiple comparison test. The significant test was considered at *P < 0.05, **P < 0.01, ***P < 0.001.

RESULTS

Table I shows effect of *Eruca sativa* aqueous extract (ESAE) on liver function tests, renal function tests and oxidative stress parameters of liver and kidney of wistar rats treated with different doses of xylene.

Table I. Effect of *Eruca sativa* aqueous extract on liver function, renal function and oxidative stress parameters of liver and kidney of wistar rats treated with different doses of xylene.

	Control			Xylene treatment		ESAE treatment	
	Tap water C	Corn oil CO	ESAE RE	1st dose X1	2nd dose X2	REX1	REX2
Liver function							
AST (µl/L)	125.8 ± 2.32	123.5 ± 5.92	121.8 ± 6.82	181.3 ± 4.11^{abc***}	189.3 ± 3.75^{abc***}	138.5 ± 4.13 ^{de***}	142.0 ± 4.81 ^{de***}
ALT (µl/L)	29.75 ± 2.29	29.5 ± 1.71	27 ± 1.64	42.25 ± 1.38^{abc**}	48.2 ± 1.53^{abc***}	32.6 ± 1.6 ^{de***}	33.4 ± 1.435 ^{de***}
ALP (µl/L)	103.3 ± 2.14	103 ± 1.82	99.75 ± 1.25	156.6 ± 2.14^{abc***}	172.3 ± 2.89$^{abc***, d**}$	117.3 ± 3.04 $^{abc*, de***}$	124.3 ± 2.29 $^{abc**, de***}$
Kidney function							
Urea (g/L)	0.32 ± 0.02	0.33 ± 0.02	0.297 ± 0.01	0.48 ± 0.03^{abc***}	0.56 ± 0.01$^{abc***, d*}$	0.32 ± 0.01^{de***}	0.39 ± 0.01$^{d*, e***}$
Uric acid(mg/L)	12.01 ± 0.40	12.06 ± 0.33	11.2 ± 0.24	15.53 ± 0.21^{abc***}	16.23 ± 0.22^{abc***}	13.52 ± 0.19$^{d**, e***}$	14.11 ± 0.09$^{d**, e***}$
Creatinine (mg/L)	7.13 ± 0.30	6.8 ± 0.45	6.75 ± 0.32	9.46 ± 0.17^{abc***}	9.96 ± 0.34^{abc***}	7.59 ± 0.22^{de***}	7.72 ± 0.29$^{d*, e***}$
Oxidative stress parameters in liver							
MDA (nmol/mg)	0.31 ± 0.01	0.29 ± 0.02	0.28 ± 0.01	0.46 ± 0.02^{abc***}	0.65 ± 0.03^{abcd***}	0.37± 0.01^{de***}	0.45 ± 0.01^{e***}
GSH (nmol/mg)	34.19 ±0.77	34.19 ±0.77	35.81 ±0.71	29.64 ± 0.47^{abc***}	27.42 ± 0.36 ^{abc***}	32.62 ± 0.51^{de**}	31.82 ± 0.42^{e***}
GPX (nmol GSH/ mg)	0.43 ± 0.008	0.42 ± 0.01	0.44 ± 0.009	0.29 ± 0.01^{abc***}	0.26 ± 0.006^{abc***}	0.34 ± 0.004$^{abc***, e***}$	0.32 ± 0.004$^{abc***, e***}$
Oxidative stress parameters in kidney							
MDA (nmol/mg)	0.32 ± 0.02	0.31 ± 0.02	0.28 ± 0.009	0.49 ± 0.02^{abc***}	0.65 ± 0.02^{abcd***}	0.38 ± 0.01$^{d**, e***}$	0.445 ± 0.01 $^{abc***, e***}$
GSH (nmol/mg)	33.27 ± 0.50	33.15 ± 0.55	34.27 ± 0.35	28.91 ± 0.42^{abc***}	26.82 ± 0.69^{abc***}	31.45 ± 0.55$^{d*, e***}$	30.46 ± 0.55^{e***}
GPX (nmol GSH/ mg)	0.41 ± 0.004	0.41 ± 0.005	0.42 ± 0.006	0.31 ± 0.007^{abc***}	0.28 ± 0.003$^{abc***, d*}$	0.34 ± 0.003$^{abc***, de***}$	0.31 ± 0.006$^{abc***, e**}$

Corn oil (0.3ml); ESAE, 350mg/kgbw; X_1, 400mg/kg bw; X_2, 800mg/kg bw; REX1, 350mg ESAE/kg body wt+ 400mg xylene/ kg bw; REX2, 350mg ESAE/kg body wt+ 800mg xylene/ kg body bw.

a, comparison with control group (C); b, comparison with CO group; c, comparison with RE group; d, comparison with 1st dose of xylene (X1); e, comparison with 2nd dose of xylene (X2). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Changes in hepatic function

The xylene-intoxicated rats (X1 and X2), showed a significant increase (P<0.001) in the AST, ALT and ALP enzymatic activities compared to rats of C, CO and RE groups. However, a significant decrease (P<0.001) was observed in animals treated with the combination of xylene and aqueous rocket extract (REX1 and REX2) comparatively to X1 and X2 groups (Table I).

Changes in renal function

A significant elevation (P<0.001) in urea, uric acid and creatinine plasma levels was reported in xylene-exposed rats (X1 and X2) in comparison with rats of C, CO and RE groups. While, the administration of plant extract combined with xylene significantly decreased (P<0.001) the levels of these metabolites in REX1 group compared to the toxic groups, and in REX2 group (P<0.05, P<0.001) relative to the X1 and X2 groups, respectively (Table I).

Changes in oxidative stress parameters in the liver

Table I shows a significant increase (P<0.001) in hepatic MDA levels in xylene-treated rats (X1 and X2) compared to the C, CO, and RE groups, and a remarkable increase (P<0.001) in the X2 group than that of the X1 group. However, ESAE + xylene administration significantly reduced (P<0.001) the increase of hepatic MDA levels in the REX1 group compared to the X1 and X2 groups, and in the REX2 group compared to the X2 group.

Treatment of rats with xylene (X1 and X2) also induced a significant decrease (P<0.001) in hepatic GSH content and GPx enzyme activity comparatively to the C, CO, and RE groups. In contrast, a significant increase (P<0.01) of hepatic GSH level was recorded in the REX1 group compared to the xylene-exposed groups (X1 and X2), and (P<0.001) in the REX2 group compared to the X2 group. There was also an improvement of GPx enzyme activity in rats treated with ESAE + xylene combination (REX1 and REX2) compared to the X2 group (Table I).

Changes in oxidative stress parameters in the kidney

The results show a significant increase (P<0.001) in renal MDA level in xylene-exposed rats (X1, X2). While,

the combined treatment of xylene+ ESAE significantly reduced (P<0.001) the renal MDA level in REX1 group compared to the X1 and X2 groups, and in REX2 group relative to the X2 group. The GSH dosage show that xylene treatment resulted in a significant decrease (P<0.001) in renal glutathione level and GPx activity when compared with the C, CO, and RE groups.

On the contrary, ESAE + xylene administration (REX1) significantly increased (P<0.001) GSH levels and GPx activity compared to the X1 and X2 groups, and considerably increased (P<0.001) in REX2 compared to the X2 group (Table I).

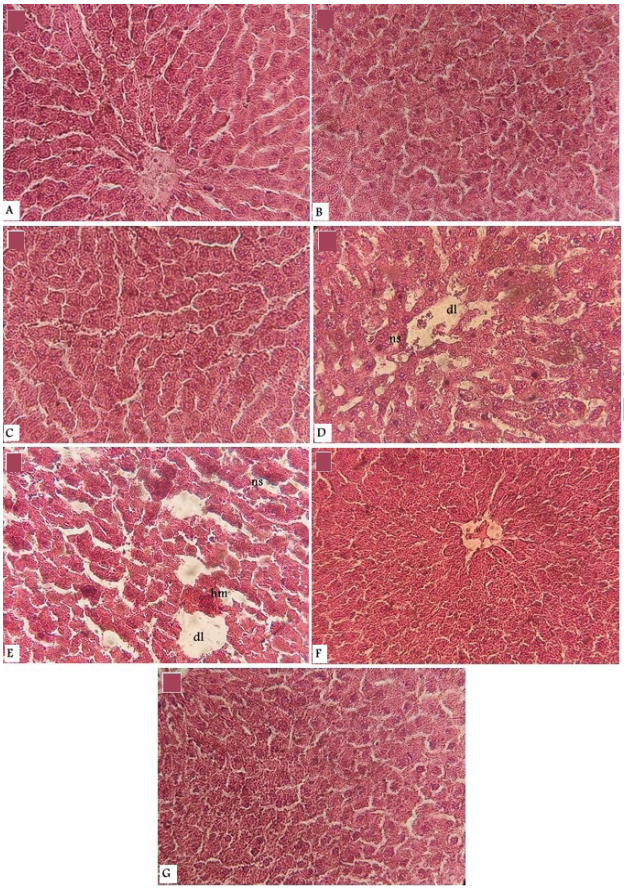

Fig. 1. Effect of *Eruca sativa* aqueous extract on liver histological structure of rats treated with different concentrations of xylene. (A) Control group, (B) CO group, (C) group treated with *Eruca sativa*, (D and E) groups exposed to xylene at 400 and 800mg/kg pc respectively, (F and G) groups treated with the combination of xylene and ESAE (H and E stain, × 400). dl, sinusoidal dilatation, hm, hemorrhage, ns, hepatocyte necrosis.

Histopathological studies

Microscopic observation of liver histological sections revealed a normal hepatic structure in the control group, CO group and the RE group treated with *Eruca sativa* extract only (Fig. 1A, B, C). Whereas in the X1 and X2 groups that received 400 and 800mg/kg pc of xylene, respectively (Fig. 1D, E) showed the degenerative changes in the liver essentially represented by sinusoidal dilatation, hemorrhage and hepatocyte necrosis. These hepatic damages induced by xylene were drastically reduced when the rocket was added to xylene (REX1 and REX2) (Fig. 1F, G).

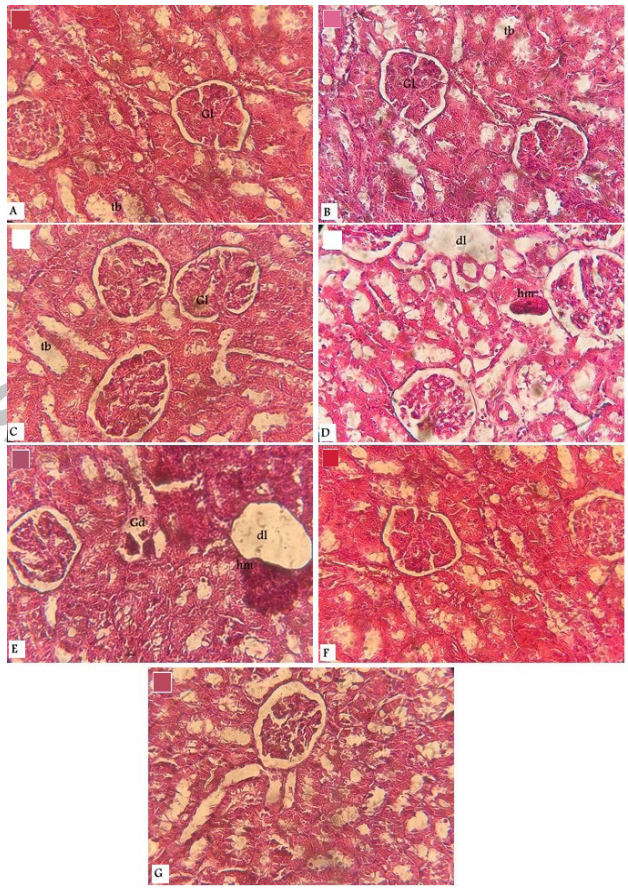

Fig. 2. Effect of *Eruca sativa* aqueous extract on kidney histological structure of rats treated with different concentrations of xylene. (A) Control group, (B) CO group, (C) group treated with *Eruca sativa*, (D and E) groups exposed to xylene at 400 and 800mg/kg pc respectively, (F and G) groups treated with the combination of xylene and ESAE (H and E stain, ×400). Gl, glomerulus, tb, tubule, dl, dilatation, hm, hemorrhage, Gd, glomerular degeneration.

Microscopic observation of kidney tissues showed a normal renal parenchyma with well-defined glomeruli and tubules in the control group, CO group and the RE group (Fig. 2A, B, C). The toxic effect of xylene on the kidney

tissue was characterized by tubular dilatation, hemorrhage and glomerular degeneration (Fig. 2D, E). However, in the REX1 and REX2 groups we observed histological ameliorations characterized by a decreased of hemorrhage and tubular dilatation, and regeneration of glomerulus compared to the X1 and X2 groups (Fig. 2F, G).

DISCUSSION

The results obtained in this study showed a significant increase of ALT, AST and ALP enzyme activity accompanied with degenerative alterations in the liver tissue mainly represented by sinusoidal dilatation, hemorrhage and hepatocyte necrosis in rats exposed to xylene. These results are in accord with the study of Neghab *et al*. (2015), who reported that levels of ALT, AST and direct bilirubin were significantly higher in the group of workers exposed to BTX (benzene, toluene and xylene) than in the control group. Ketan *et al*. (2013) also reported high levels of liver transaminases (ALT and AST) in mice exposed to toluene, xylene, and benzene mixtures. Furthermore, our results are consistent with that of Yoon and Lee (2010), which showed a significant increase in ALT, AST and LDH levels with histopathological changes in the liver of xylene-treated rats including moderate hepatic necrosis and lobule inflammation. Similar observations were made by Dere and Ari (2008), have revealed that benzene affected four important liver markers (ALT, AST, ALP and LDH) in the serum of rats.

In accordance with our study, Doherty *et al*. (2019) have reported histological alterations in the liver of the fish (*Clarias gariepinus*) after exposure to high sublethal concentrations of xylene. These pathological changes included severe hepatic necrosis with spotted structures, inflammation, vacuolization and bile stagnation. In another study, microscopic evaluation of liver tissue of toluene-exposed rats revealed dilated sinusoids with hemorrhage (Kanter, 2011).

Many studies have confirmed that increased in ALT and AST enzyme activities indicating hepatic necrosis (Ann *et al*., 2001). Similarly, Rahman *et al*. (2000) suggested that elevated ALP plasma levels could be due to increased plasma membrane permeability or cell necrosis. These hepatotoxic effects of xylene seem to be related to the elevated production of caspase-3 and caspase-9 conducting the apoptosis, as well as an increase in CYP2E1 activity (necrosis mediator) (Al-Ghamdi *et al*., 2003a, b, 2004).

In addition, the kidney plays an important function in the organism homeostasis ensuring the filtration of toxic wastes from the blood circulation and their elimination in the urine (Alvarez-Llamas *et al*., 2012). Four nitrogen constituents, including urea, uric acid, creatinine and albumin are considered as important biomarkers of kidney toxicity (Boubchir, 2002).

In our experimental study, an increase in plasma concentrations of urea, uric acid and creatinine was noticed in rats treated with xylene. These results correlate with the research of Neghab *et al*. (2015) who have found that serum urea and creatinine levels were significantly higher in the BTX-exposed group than in the control group. Kum *et al*. (2007b) have shown that serum urea level increased in the group treated with 300 ppm of technical xylene, while creatinine activity did not show significant difference between control rats and other experimental groups. Creatinine and urea are the main markers of renal insufficiency, and uric acid is the final result of purine catabolism and is an important antioxidant in human plasma because it can react directly with free radicals (Alvarez-Lario and Macarron-Vicente, 2010).

In this study, the histopathological effect of xylene on the kidney tissue was characterized by tubular dilatation, hemorrhage and glomerular degeneration. Abouee-Mehrizi *et al*. (2020) reported that exposure to toluene at 1000±50 ppm caused glomerular cells shrinkage, glomerular congestion and vacuolization in rabbit kidney. The results of Meydan *et al*. (2013) also showed capsule differentiation, shrinkage of glomerular tufts, and increased connective tissue in the renal interstitial area in wistar rats after exposure to 500 mg/kg of toluene.

Revilla *et al*. (2007) found that xylene was capable of causing an important mitochondrial swelling, apparently associated with increased ROS generation. The high production of ROS acts as effectors of necrosis by inducing oxidative lesions directly to the cell and its components. But a low level of oxidation influences apoptosis during the engagement phase by functioning as signaling intermediates or as activators of caspases (Finkel, 1998). Oxidative stress alters the function of glomerulus more than other parts of the nephron (Yi *et al*., 2011).

Some researchers have reported that organic solvents induce the alterations in the antioxidant system, notably the activity of superoxide dismutase (SOD), GPx and the blood content of GSH and MDA in petrochemical industry workers (Croute *et al*., 2002; Georgieva *et al*., 2002; Singh *et al*., 2009).

In the present study, we observed a significant increase in MDA levels accompanied by significant reduction of GSH levels and GPx activity in the liver and kidney of rats subjected to xylene. These data are consistent with a previous study by Singh *et al*. (2010) who confirmed a significant increase in MDA content with GSH depletion in *Drosophila* exposed to xylene. In another study. Kum *et al*. (2007a) also found an elevated level of hepatic MDA

associated with a decrease in GSH levels after xylene inhalation. In contrast, GPx activity and the levels of GSH and MDA showed a significant increase in the renal tissue of rats (Kum *et al*., 2007b).

MDA is a principal product of free radical damage on polyunsaturated fatty acids and it is used extensively as a biomarker of lipid peroxidation (Lasheras *et al*., 2002). The MDA elevation induced by xylene may be a consequence of altered antioxidant defense systems such as GSH and GSH-related enzymes (Salimi *et al*., 2017). Oxidative stress conducts to the formation of electrophilic intermediates capable of reacting with the sulfhydryl group and leading GSH depletion (Snyder and Hedli, 1996; Croute *et al*., 2002). The observed reduction in GPx activity can lead to accumulation of hydrogen peroxide (H_2O_2) in different organs (Kamel and Shehata, 2008). H_2O_2 is mainly generated by mitochondria and diffuses into lysosomes in abnormal quantity. Since many lysosomes are rich in redox-active Fe2+/Fe3+, Fenton-type reactions then take place resulting in lysosomal membrane injury with liberation of powerful lytic enzymes (Kurz *et al*., 2008).

However, the concomitant administration of xylene and ESAE significantly reduced all the elevated biochemical parameters (ALT, AST, ALP, urea, uric acid and creatinine levels) compared to rats treated exclusively with xylene. On the other hand, there was a significant increase in GSH levels and GPx activity accompanied by a decrease in MDA levels in the liver and kidney of rats treated with xylene + ESAE compared to rats treated with xylene alone. ESAE was also effective in modifying hepatic and renal histopathological damages to semi-normal. This indicates a significant improvement in the functional status of liver and kidney due to the antioxidant activity of *Eruca sativa.*

Mashi (2017) repoted significant decrease in transaminases (ALT and AST) due to administration of aqueous extract of *E sativa* in male rabbits exposed to phosphoric acid. Similarly, El-Sadek (2014) indicated that administration of rocket (the leaves, juice, oil and seeds) caused an improvement in ALT, AST, and ALP activities in rats treated with paracetamol. Al-Qasoumi (2010) also reported that pretreatment with ethanolic extract of *E sativa* (250 and 500 mg/ kg) significantly prevented the elevation of ALT, AST and ALP induced by carbon tetrachloride and attenuated the degree of hepatic damage indicated by only mild inflammation.

Rocket is commonly used in traditional medicine as a remedy for kidney disease (Abodola *et al*., 2015). Researchers have shown that *E sativa* has a nephroprotective effect evidenced by significant decreases in serum urea, creatinine, and elevated ALP activity and an improvement of renal tubular necrosis in gentamicin-treated rats (Elgazar and Aboraya, 2013).

A significant decrease in ALT, AST, ALP, creatinine and urea levels with an improvement in the morphological form of the affected hepatic and renal cells (mild necrosis and inflammation) were observed in tumor mice after treatment with *Eruca sativa* seeds and leaves extracts (El-Sadek *et al*., 2021). Our observations are in accordance with other research by Kamil *et al*. (2019) who reported that *Eruca sativa* oil has a reparative action on UV-damaged kidney and liver tissues in white mice, by increasing the regenerative processes in these organs through activation of hepatic and renal progenitor cells (stem cells).

Alam *et al*. (2007) demonstrated that treatment of rats with *E sativa* seed extract prior to treatment with mercuric chloride ($HgCl_2$) resulted in recovery of reduced levels in all antioxidant enzymes (glutathione reductase (GR), Catalase (CAT), SOD, GSH and GPx), and inhibition of lipid peroxidation in the kidney. In the same line, El-Sadek (2014) revealed a significant reduction in lipid peroxidation caused by paracetamol intoxication, by a decrease of hepatic MDA level as well as an increase of antioxidant enzymes including glutathione S-transferase (GST), SOD and GPX in the liver of rats treated with rocket.

The hepatoprotective effect of *E sativa* may be due to inhibition of the cytochrome P450 oxygenase enzyme system (Hanlon *et al*., 2008). On the other hand, glucosinolates are known to be metabolized to isothiocyanates (ITC) that induce the metabolizing enzymes of phase II (GST, epoxide hydrolase, NADPH: quinone reductase) which plays an important role in electrophiles detoxification and protection against oxidative stress (Fahey and Talalay, 1999). Particular glucosinolate is a sulfur compound contains asymmetric S-S links that donor electrons to free molecules (Kim and Ishii, 2006). The principal glucosinolate in rocket is glucoerucine, which has hydroperoxide decomposition properties such as alkyl hydroperoxide and H_2O_2 (Barillari *et al*., 2005).

In previous studies, the renal protective effect has been linked to the presence of polyunsaturated fatty acids, phenolic compounds, and phytosterols that are free radical scavengers because they have antioxidant and anti-inflammatory properties (Al-Okbi *et al*., 2014). *E sativa* extract is able to maintain levels of antioxidant molecules and antioxidant enzymes in the kidney and protect renal tissue from oxidative damage (Alam *et al*., 2007).

CONCLUSION

In conclusion, the results of this study indicate that the ESAE was effective in the prevention of xylene-induced liver and kidney damage in male wistar rats. These hepato

and nephroprotective properties of *Eruca sativa* may be due to both an increase in antioxidant enzymes activity and to the inhibition of lipid peroxidation.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abodola, M.A., Lutfi, M.F., Bakhiet, A.O. and Mohamed, A.H., 2015. The anti-edema effect of *Eruca sativa* seeds. *J. Sci*., **5**: 1340-1344.

Abouee-Mehrizi, A., Rasoulzadeh, Y., Mesgari-Abbasi, M., Mehdipour, A. and Ebrahimi-Kalan, A., 2020. Nephrotoxic effects caused by co-exposure to noise and toluene in New Zealand white rabbits: A biochemical and histopathological study. *Life Sci.,* pp. 118254. https://doi.org/10.1016/j.lfs.2020.118254

Agency for Toxic Substances and Disease Registry (ATSDR), 2007. *Toxicological profile for xylenes*. Department of Health and Human Services, Public Health Service, Atlanta, GA: U.S.

Al-Qasoumi, S., 2010. Carbon tetrachloride-induced hepatotoxicity: Protective effect of "Rocket" *Eruca sativa L.* in rats. *Am. J. Chin. Med*., **38**: 75–88. https://doi.org/10.1142/S0192415X10007671

Alam, M.S., Kaur, G., Jabbar, Z., Javed, K. and Athar, M., 2007. *Eruca sativa* seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. *Fd. Chem. Toxicol*., **45**: 910–920. https://doi.org/10.1016/j.fct.2006.11.013

Al-Ghamdi, S.S., Raftery, M.J. and Yaqoob, M.M., 2003a. Acute solvent exposure induced activation of cytochrome P4502E1 causes proximal tubular cell necrosis by oxidative stress. *Toxicol. in Vitro*, **17**: 335–341. https://doi.org/10.1016/S0887-2333(03)00021-3

Al-Ghamdi, S.S., Raftery, M.J. and Yaqoob, M.M., 2003b. Organic solvent-induced proximal tubular cell toxicity via caspase-3 activation. *J. Toxicol. clin. Toxicol.,* **41**: 941–945. https://doi.org/10.1081/CLT-120026515

Al-Ghamdi, S.S., Raftery, M.J. and Yaqoob, M.M., 2004. Organic solvent-induced proximal tubular cell apoptosis via caspase-9 activation. *Environ. Toxicol. Pharmacol*., **16**: 147–152. https://doi.org/10.1016/j.etap.2003.12.002

Al-Okbi, S.Y., Mohamed, D.A., Hamed, T.E., Esmail, R.S. and Donya, S.M., 2014. Prevention of renal dysfunction by nutraceuticals prepared from oil rich plant foods. *Asian Pac. J. trop. Biomed*., **4**: 618–626. https://doi.org/10.12980/APJTB.4.201414B66

Alvarez–Lario, B. and Macarron-Vicente, J., 2010. Uric acid and evolution. *Rheumatology,* **49**: 2010–2015. https://doi.org/10.1093/rheumatology/keq204

Alvarez-Llamas, G., Zubiri, I., Maroto, A.S., De la Cuesta, F., Posada-Ayala, M., Martin-Lorenzo, M. and Vivanco, F., 2012. A role for the membrane proteome in human chronic kidney disease erythrocytes. *Transl. Res.,* **160**: 374-383. https://doi.org/10.1016/j.trsl.2012.06.004

Ann, C.Y., Lee, K.J., Park, J.B., Jang, J.Y. and Kim, M.J., 2001. The association of exposure to organic solvents with liver function. *Korean J. Occup. environ. Med.*, **13**: 64–74. https://doi.org/10.35371/kjoem.2001.13.1.64

Barillari, J., Canistro, D., Paolini, M., Ferroni, F., Pedulli, G.F., Iori, R. and Valgimigli, L., 2005. Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca sativa Mill.)* seeds and sprouts. *J. Agric. Fd. Chem.,* **53**: 2475–2482. https://doi.org/10.1021/jf047945a

Boubchir, M.A., 2002. Biochimie de la nephrologie. 2 EME ed. Alger: Opu, pp. 320. ISBN 00-789-23-320.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72**: 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brater, D.C. and Hall, S.D., 2000. Disposition and dose requirements of drugs in renal insufficiency. In: *The kidney physiology and pathophysiology* (eds. D.W. Seldin, G. Giebisch and Lippincott), 3. Williams and Wilkins, Philadelphia. pp. 2923–2942.

Chen, K., Gunter, K. and Maines, M.D., 2000. Neurons overexpressing heme oxygenase-1 resist oxidative stress-mediated cell death. *J. Neurochem.,* **75**: 304–313. https://doi.org/10.1046/j.1471-4159.2000.0750304.x

Croute, F., Poinsot, J., Gaubin, Y., Beau, B., Simon, V., Murat, J.C. and Soleilhavoup, J.P., 2002. Volatile organic compounds cytotoxicity and expression of HSP72, HSP90 and GRP78 stress proteins in cultured human cells. *Biochim. biophys. Acta Mol. Cell Res*., **1591**: 147–155. https://doi.org/10.1016/S0167-4889(02)00271-9

Dere, E. and Ari, F., 2008. Effect of benzene on liver functions in rats (*Rattus norvegicus*). *Environ. Monit. Assess.,* **154**: 23–27. https://doi.org/10.1007/s10661-008-0374-7

Doherty, V.F., Adeola, A., Aneyo, A.I. and Otitoloju, A.A., 2019. Histopathological biomarkers of exposure to monocyclic aromatic hydrocarbons in *Clarias gariepinus* (African Catfish). *J. Sci. Technol*., **11**: 34-44.

El-Sadek, M.F., 2014. Chemical constituents of *Eruca sativa* and treatment activity against paracetamol inducing hepatic injury in experimental rats. *J. Nutr. Hlth*., **9**: 73-84. https://doi.org/10.21608/ejnh.2014.4817

El-Sadek, M.F., Essam El-Din, M.M. and Ahmed, B.M., 2021. Evaluation of anticarcinogenic and antioxidant properties of *Eruca sativa* extracts versus Ehrlich ascites carcinoma in mice. *J. King Saud Univ. Sci.*, **33**: 101435. https://doi.org/10.1016/j.jksus.2021.101435

Elgazar, A.F. and Aboraya, A.O., 2013. Nephroprotective and diuretic effect of three medicinal herbs against gentamicin-induced nephrotoxicity in male rats. *Pak. J. Nut*., **12**: 715-722. https://doi.org/10.3923/pjn.2013.715.722

Fahey, J.W. and Talalay, P., 1999. Antioxidant functions of sulforaphane: a potent inducer of Phase II detoxication enzymes. *Fd. Chem. Toxicol*., **37**: 973–979. https://doi.org/10.1016/S0278-6915(99)00082-4

Finkel, T., 1998. Oxygen radicals and signaling. *Curr. Opin. Cell Biol.,* **10**: 248–253. https://doi.org/10.1016/S0955-0674(98)80147-6

Flohé, L. and Günzler, W.A., 1984. Assays of glutathione peroxidase. *Methods Enzymol*., **105**: 114-121. https://doi.org/10.1016/S0076-6879(84)05015-1

Garg, G. and Sharma, V., 2014. *Eruca sativa* (L.): Botanical description, crop improvement, and medicinal properties. *J. Herbs Spices Med. Pl.,* **20**: 171–182. https://doi.org/10.1080/10496475.2013.848254

Georgieva, T., Michailova, A., Panev, T. and Popov, T., 2002. Possibilities to control the health risk of petrochemical workers. *Int. Arch. Occup. environ. Hlth*., **75**: 21–26. https://doi.org/10.1007/s00420-002-0344-2

Hanlon, N., Coldham, N., Sauer, M.J. and Ioannides, C., 2008. Up-regulation of the CYP1 family in rat and human liver by the aliphatic isothiocyanates erucin and sulforaphane. *Toxicology,* **252**: 92–98. https://doi.org/10.1016/j.tox.2008.08.002

Hould, R., 1984. *Techniques d'histopathologie et de cytopathologie*. Ed Maloine., pp. 19-21.

Kanter, M., 2011. Protective effect of quercetin on liver damage induced by chronic toluene exposure in rats. *Toxicol. Ind. Hlth.,* **28**: 483 491. https://doi.org/10.1177/0748233711414613.

Kamel, E.N. and Shehata, M., 2008. Effect of toluene exposure on the antioxidant status and apoptotic pathway in organs of the rat. *Br. J. biomed. Sci*., **65**: 75–79. https://doi.org/10.1080/09674845.2008.11732801

Kamil, A.M., Hussain, A.H.M., Kadoori, Y.T. and Ahmad, S.J., 2019. Eruca sativa and *Raphanus sativus* oils enhance hepatic and renal tissues regeneration in white mice. *Al-Mustansiriyah. J. Sci.,* **29**: 27–37. https://doi.org/10.23851/mjs.v29i4.404

Kasote, D.M., Katyare, S.S., Hegde, M.V. and Bae, H., 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *Int. J. biol. Sci.,* **11**: 982–991. https://doi.org/10.7150/ijbs.12096

Ketan, V.K., Bhavyata, K., Linzbuoy, G. and Hyacinth, H.N., 2013. Renal and hepatotoxic alterations in adult mice on inhalation of specific mixture of organic solvents. *Toxicol. Ind. Hlth.,* **31**: 1158–1164. https://doi.org/10.1177/0748233713485892

Kim, S.J. and Ishii, G., 2006. Glucosinolate profiles in the seeds, leaves and roots of rocket salad (*Eruca sativa Mill*.) and anti-oxidative activities of intact plant powder and purified 4-methoxyglucobrassicin. *Soil Sci. Pl. Nutr*., **52**: 394–400. https://doi.org/10.1111/j.1747-0765.2006.00049.x

Kim, S.J., Jin, S. and Ishii, G., 2004. Isolation and structural elucidation of 4-(β-D Glucopyranosyldisulfanyl) butyl glucosinolate from leaves of rocket salad (*Eruca sativa L.)* and its antioxidative activity. *Biosci. Biotechnol. Biochem.,* **68**: 2444-2450. https://doi.org/10.1271/bbb.68.2444

Kum, C., Kiral, F., Sekkin, S., Seyrek, K. and Boyacioglu, M., 2007a. Effects of xylene and formaldehyde inhalations on oxidative stress in adult and developing rats livers. *Exp. Anim.,* **56**: 35–42. https://doi.org/10.1538/expanim.56.35

Kum, C., Sekkin, S., Kiral, F. and Akar, F., 2007b. Effects of xylene and formaldehyde inhalations on renal oxidative stress and some serum biochemical parameters in rats. *Toxicol. Ind. Hlth.,* **23**: 115–120. https://doi.org/10.1177/0748233707078218

Kurz, T., Terman, A., Gustafsson, B. and Brunk, U.T., 2008. Lysosomes and oxidative stress in aging and apoptosis. *Biochim. biophys. Acta*, **1780**: 1291-1303. https://doi.org/10.1016/j.bbagen.2008.01.009

Lasheras, C., Huerta, J.M., Gonzalez, S., Braña, A.F., Patterson, A.M. and Fernandez, S., 2002. Independent and interactive association of

blood antioxidants and oxidative damage in elderly people. *Free Radic. Res.,* **36**: 875-882. https://doi.org/10.1080/1071576021000005311

Le-Floch, S., Aprin, L., Fuhrer, M. and Slangen, P., 2012. Environmental parameter effects on the fate of a chemical slick. In: *Air quality monitoring and modeling* (eds. S. Kumar), Intech Open. https://doi.org/10.5772/32950

Malaguarnera, G., Cataudella, E., Giordano, M., Nunnari, G., Chisari, G. and Malaguarnera, M., 2012. Toxic hepatitis in occupational exposure to solvents. *World J. Gastroenterol*., **18**: 2756-2766. https://doi.org/10.3748/wjg.v18.i22.2756

Mashi, S.K., 2017. Effect of *Eruca sativa* leaves extract on liver enzymes and lipid profile in phosphoric acid induced liver damage in male rabbits. *J. Ent. Zool. Stud*., **5**: 1011-1015.

Meydan, S., Nacar, A., Oztürk, H.O., Tas, U., Köse, E., Zararsiz, I., and Kus, I., 2013. The protective effects of caffeic acid phenethyl ester against toluene-induced nephrotoxicity in rats. *Toxicol. Ind. Hlth.,* **32**: 15–21. https://doi.org/10.1177/0748233713485890

Neghab, M., Hosseinzadeh, K. and Hassanzadeh, J., 2015. Early liver and kidney dysfunction associated with occupational exposure to sub-threshold limit value levels of benzene, toluene, and xylenes in unleaded petrol. *Saf. Hlth. Work,* **6**: 312-316. https://doi.org/10.1016/j.shaw.2015.07.008

Ohkawa, H., Ohishi, N. and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*., **95**: 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Rahman, M.F., Siddiqui, M.K. and Jamil, K., 2000. Acid and alkaline phosphatase activities in a novel phosphorothionate (RPR-11) treated male and female rats; evidence of dose and time-dependent response. *Drug Chem. Toxicol.,* **23**: 497–509. https://doi.org/10.1081/DCT-100100131

Revilla, A.S., Pestana, C.R., Pardo-Andreu, G.L., Santos, A.C., Uyemura, S.A., Gonzales, M.E. and Curti, C., 2007. Potential toxicity of toluene and xylene evoked by mitochondrial uncoupling. *Toxicol. In Vitro.,* **21**: 782-788. https://doi.org/10.1016/j.tiv.2007.01.012

Salimi, A., Talatappe, B.S. and Pourahmad, J., 2017. Xylene induces oxidative stress and mitochondria damage in isolated human lymphocytes. *Toxicol. Res.,* **33**: 233–238. https://doi.org/10.5487/TR.2017.33.3.233

Singh, M.P., Ravi Ram, K., Mishra, M., Shrivastava, M., Saxena, D.K. and Chowdhuri, D.K., 2010. Effects of co-exposure of benzene, toluene and xylene to Drosophila melanogaster: Alteration in hsp70, hsp60, hsp83, hsp26, ROS generation and oxidative stress markers. *Chemosphere*, **79**: 577–587. https://doi.org/10.1016/j.chemosphere.2010.01.054

Singh, M.P., Reddy, M.M.K., Mathur, N., Saxena, D.K. and Chowdhuri, D.K., 2009. Induction of hsp70, hsp60, hsp83 and hsp26 and oxidative stress markers in benzene, toluene and xylene exposed *Drosophila melanogaster*: Role of ROS generation. *Toxicol. appl. Pharmacol*., **235**: 226–243. https://doi.org/10.1016/j.taap.2008.12.002

Snyder, R. and Hedli, C.C., 1996. An overview of benzene metabolism. *Environ. Hlth. Perspect.,* **104**: 1165–1171. https://doi.org/10.1289/ehp.961041165

Vieira, J.F., Oliveira, S.D., Zanatta, Z.C.N., Lemes, E.S., Villela, F.A. and Barros, A.C.S.A., 2015. Physiological and phytosanitary potential of rocket seeds. *Ciência Rural*, **45**: 200–205. https://doi.org/10.1590/0103-8478cr20130728

Weckbecker, G. and Cory, J.G., 1988. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. *Cancer Lett*., **40**: 257–264. https://doi.org/10.1016/0304-3835(88)90084-5

Yehuda, H., Khatib, S., Sussan, I., Musa, R., Vaya, J. and Tamir, S., 2009. Potential skin anti-inflammatory effects of 4-methylthiobutylisothiocyanate (MTBI) isolated from rocket (*Eruca sativa*) seeds. *BioFactors,* **35**: 295–305. https://doi.org/10.1002/biof.32

Yi, X., Nickeleit, V., James, L.R. and Maeda, N., 2011. α-Lipoic acid protects diabetic apolipoprotein E-deficient mice from nephropathy. *J. Diab. Complic.,* **25**: 193–201. https://doi.org/10.1016/j.jdiacomp.2010.07.004

Yoon, I.S. and Lee, H.J., 2010. Effect of liver damage by combined administration of ethanol and xylene in rats. *J. Korea. Contents Assoc.*, **10**: 259-267. https://doi.org/10.5392/JKCA.2010.10.7.259